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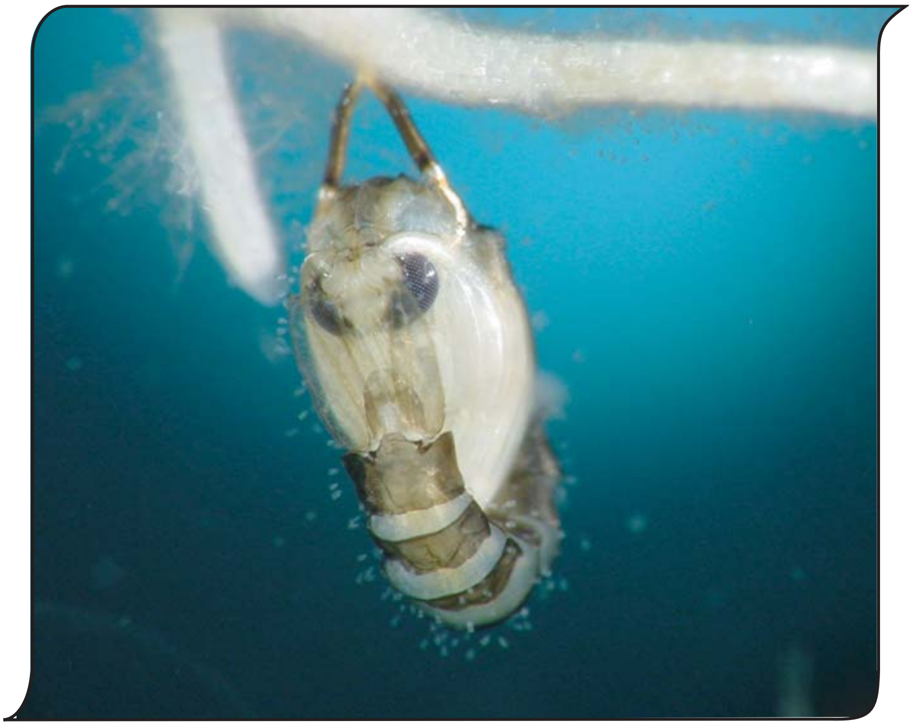
THE NEW SOUTH WALES
ARBOVIRUS SURVEILLANCE &
MOSQUITO MONITORING PROGRAM

2003 - 2004
Annual Report



Coquillettidia linealis pupa

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NSW HEALTH

Authorised by
ARBOVIRUS DISEASE CONTROL ADVISORY GROUP, NSW HEALTH.

EXECUTIVE OVERVIEW

- For the 2003-2004 season, the NSW Arbovirus Surveillance Program: (i) Monitored mosquito vector populations and undertook surveillance of arbovirus activity on the NSW western slopes and plains, far north coast region and metropolitan Sydney. (ii) Monitored flavivirus transmission through the testing of sentinel chickens across inland NSW. The majority of sites operated between November and April.

- The dry conditions during the 2002-2003 season continued for much of 2003-2004. Despite above average precipitation for the southern inland late in 2003, little rain fell during the first six months of 2004 and mosquito numbers were again well below average for the inland.

- With the low mosquito numbers, there was minimal arbovirus activity, with only 13 isolates (2 Ross River (RRV), 1 Edge Hill (EHV), 4 Kokobera (KOKV) and 6 unknown viruses) from the mosquitoes collected inland. Arbovirus notifications from the inland region (202) were higher this season compared to the previous two, although down upon the previous five season average of 262.

- Despite the dry conditions, the relatively low mosquito and arbovirus activity, there was one seroconversion to Murray Valley encephalitis virus (MVEV) in the sentinel chickens. This occurred at Menindee from the blood collection taken on 23/Dec/2003, after a period of localised rainfall.

- Only five coastal locations again undertook trapping this year. Coastal rainfall patterns were below average for much of the first half of 2004, although some heavy rainfall occurred during February along the north coast. This led to some large mosquito collections, especially from Port Stephens, and so overall mosquito numbers for the coast were up this season.

- Barmah Forest virus (BFV) was again active along the north coast for the fourth season running, with most disease notifications occurring within the Northern Rivers (125 cases) and Mid-North Coast AHSs (149). These regions produced almost 80% of all BFV reports for NSW. Analysis of the local government areas of the north coast shows that Port Macquarie has produced the highest numbers of BFV disease cases for NSW over the last ten seasons. RRV was also active in these two AHSs, with a total of 305 cases out of the state total of 641 notifications.

- For the Sydney trapping locations, mosquito numbers were relatively low with the dry summer. Likewise human notifications from the region were lower than average.

- The NSW Arbovirus Surveillance Web Site http://www.arbovirus.health.nsw.gov.au/ continued to expand, and now has over 155MB of information with 1,140 pages and receives around 23,000 hits/month.

TABLE OF CONTENTS

NSW ARBOVIRUS SURVEILLANCE AND MOSQUITO MONITORING PROGRAM 2003-2004

INTRODUCTION

The aim of the Program is to provide an early warning of the presence of Murray Valley encephalitis virus (MVEV) and Kunjin (KUNV) viruses in the state in an effort to reduce the potential for human disease. In addition, the Program compiles and analyses mosquito and alphavirus, especially Ross River (RRV) and Barmah Forest (BFV) viruses, data collected over a number of successive years. This will provide a solid base to determine the underlying causes of the seasonal fluctuations in arbovirus activity and the relative abundance of the mosquito vector species with the potential to affect the well being of human communities. This information can then be used as a basis for modifying existing local and regional vector control programs, and in the creation of new ones.

METHODS

Background

Arbovirus activity within NSW has been defined by the geography of the state and three broad virogeographical zones are evident: the inland, the tablelands and the coastal strip (Doggett 2005). Within these zones there are different environmental influences (e.g. irrigation provides a major source of water for mosquito breeding inland, while saltmarshes along the coast are highly productive), different mosquito vectors, different viral reservoir hosts and even different mosquito borne viruses (e.g. MVEV and KUNV occur only in the inland, while BFV is active mainly on the coast). As a consequence, arboviral disease epidemiology of is often vastly different and thus the surveillance program is tailored around these variables.

Arbovirus surveillance can be divided into two categories: those methods that attempt to predict activity and those that demonstrate viral transmission. Predictive methods include the monitoring of weather patterns, the long-term recording of mosquito abundance, and the isolation of virus from vectors. Monitoring of rainfall patterns, be it short term with rainfall or longer term with the Southern Oscillation, is critical as rainfall is one of the major environmental factors that influences mosquito abundance; generally the more rain, the higher the mosquito numbers. The long-term recording of mosquito abundance can establish baseline mosquito levels for a location (i.e. determine what are normal populations), and this allows the rapid recognition of unusual mosquito activity. The isolation of virus from mosquito vectors can provide the first indication of which arboviruses are circulating in an area. This may lead to the early recognition of outbreaks and be a sign of the potential disease risks to the community. Virus isolation can also identify new viral incursions, lead to the recognition of new virus genotypes and identify new vectors. Information from vector monitoring can also reinforce and strengthen health warnings of potential arbovirus activity.

Methods that demonstrate arboviral transmission include the monitoring of suitable sentinel animals (such as chickens) for the presence of antibodies to particular viruses (e.g. MVEV and KUNV within NSW) and the recording of human cases of disease. Sentinel animals can be placed into potential 'hotspots' of virus activity, and as they are continuously exposed to mosquito bites, may indicate activity in a region before human cases are reported or before the virus has been isolated from mosquitoes. Seroconversions in sentinel flocks provide evidence that the level of virus in mosquito populations is high enough for transmission to occur.

The monitoring of human cases of arboviral infection has little direct value for surveillance, as by the time the virus activity is detected in the human population, often not much can be done to control the viral transmission. Via the other methodologies, the aim of the surveillance program is to recognise both potential and actual virus activity before it impacts greatly on the human population so that appropriate preventive measures can be implemented. The recording of human infections does however provide important epidemiological data and can define the locations where surveillance should occur in future.

These methods of surveillance are listed in order; generally with more rainfall comes more mosquito production. The higher the mosquito production, the greater the probability of enzootic virus activity in the mosquito/host population. The higher the proportion of virus infected hosts and mosquitoes, the greater the probability of transmission and thus the higher the risk to the human population. The NSW Arbovirus Surveillance and Mosquito Monitoring Program undertakes the first four methods of arbovirus surveillance and the results for the 2003-2004 season follow.

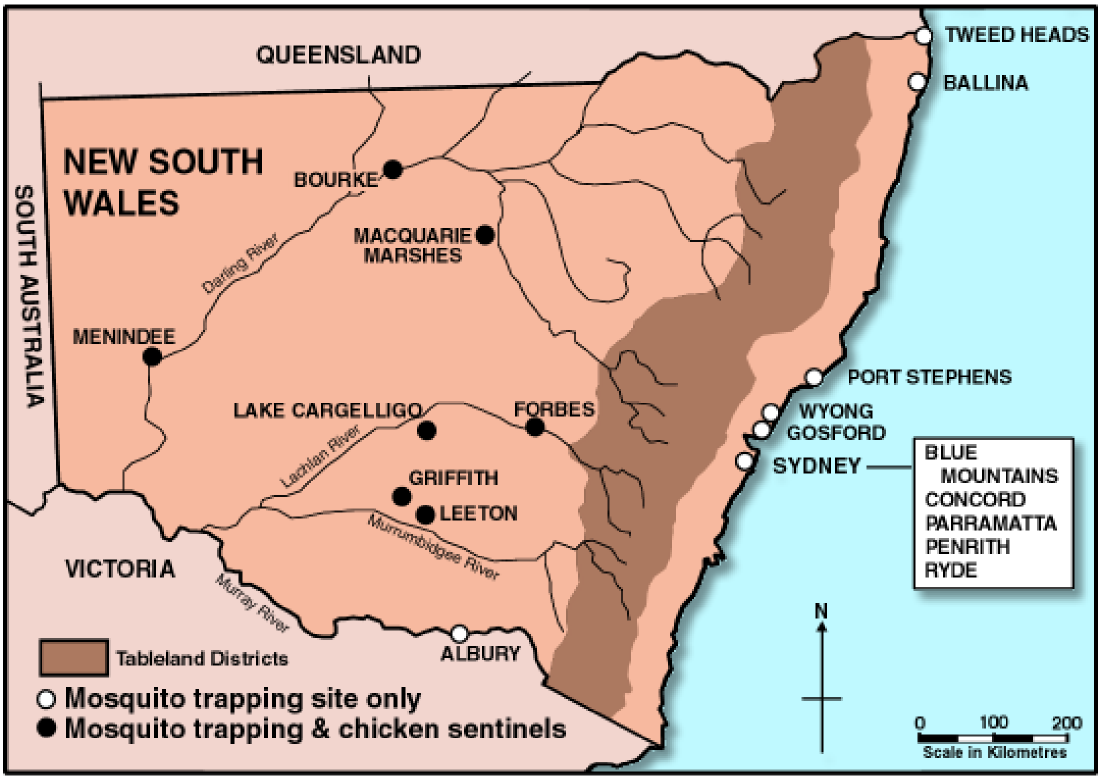


Fig 1. Mosquito trapping locations and Sentinel Chicken sites, 2003-2004.

MONITORING LOCATIONS

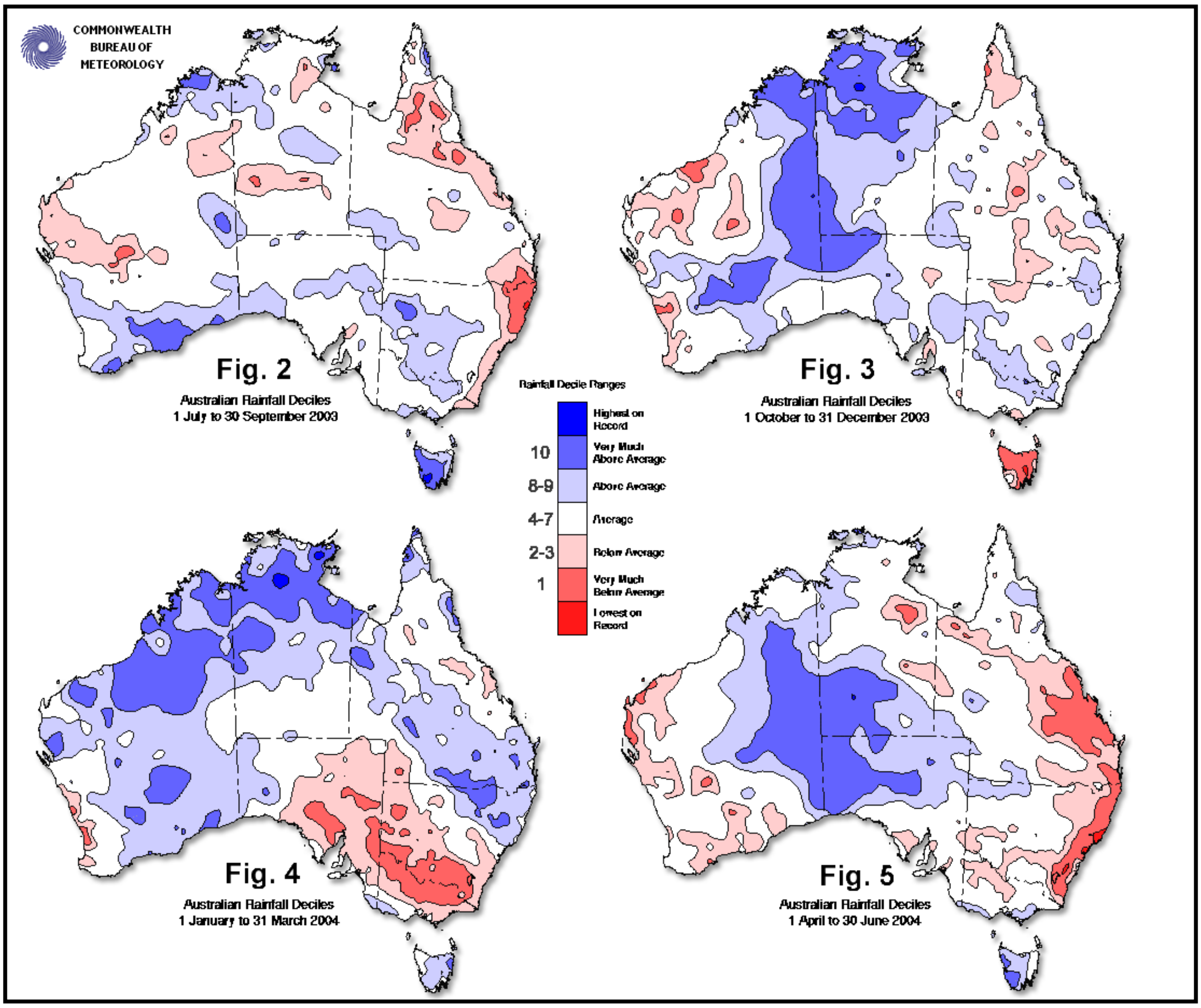
http://www.arbovirus.health.nsw.gov.au/areas/arbovirus/location/locations.htm

For 2003-2004, mosquito-trapping sites were operated at 8 inland, 5 coastal and 5 Sydney locations (Fig 1). Chicken sentinel flocks were located at 6 sites.

WEATHER DATA

http://www.arbovirus.health.nsw.gov.au/areas/arbovirus/climate/climate.htm

Mosquito abundance is dictated principally by rainfall patterns and irrigation practices in inland regions, while in coastal regions tidal inundation along with rainfall is important. Temperature and/or day-length are often critical in determining the initiation and duration of mosquito activity for species in temperate zones. Hence, the monitoring of environmental parameters, especially rainfall, is a crucial component of the Program.



Figures 2-5. Australian Rainfall deciles for the 3 month periods, Jul-Sep 2003, Oct-Dec 2003, Jan-Mar 2004 & Apr-Jun 2004. The stronger the red, the drier the conditions. Conversely, the stronger the blue, the wetter the conditions. *Modifed from the Australian Bureau of Meterology, 2004*.

The mosquito season of 2002-2003 had rainfall that was well below average, in fact the last half of 2002 was one of the driest periods ever recorded. The six months January to June of 2003 failed to improve on the drought conditions, having only average rainfall for much of the state. For the remainder of the year, the coast experienced well below average rainfall (Figures 2 & 3), while the southern inland had some wet spells resulting in slightly above average precipitation and the northern inland had average rainfall. Extremely dry conditions returned for southern inland NSW during the first half of 2004 (Figures 4 & 5), although along the mid-north coast, particularly at Port Stephens and Port Macquarie, some heavy rainfall was recorded during February (Figure 4). This was followed by a period of exceptionally dry weather for the entire coastal strip of NSW during April to June (Figure 5).

With the dry conditions, warmer weather prevailed and temperatures were well above average from November 2003 to April 2004.

MVEV Predictive Models

Two models have been developed for the prediction of MVEV outbreaks in southeastern Australia; the Forbes' (1978) and Nicholls' (1986) models.

Forbes associated rainfall patterns with the 1974 and previous MVEV endemics, and discussed rainfall in terms of 'decile' values. A decile is a ranking based on historical values. The lowest 10% of all rainfall values constitute decile 1, the next 10% make up decile 2, and so on up to the highest 10% of rainfall constituting decile 10. Thus, the higher the decile value, the greater the rainfall.

Forbes' hypothesis refers to rainfall levels in the catchment basins of the main river systems of eastern Australia. These include:

- The Darling River system,

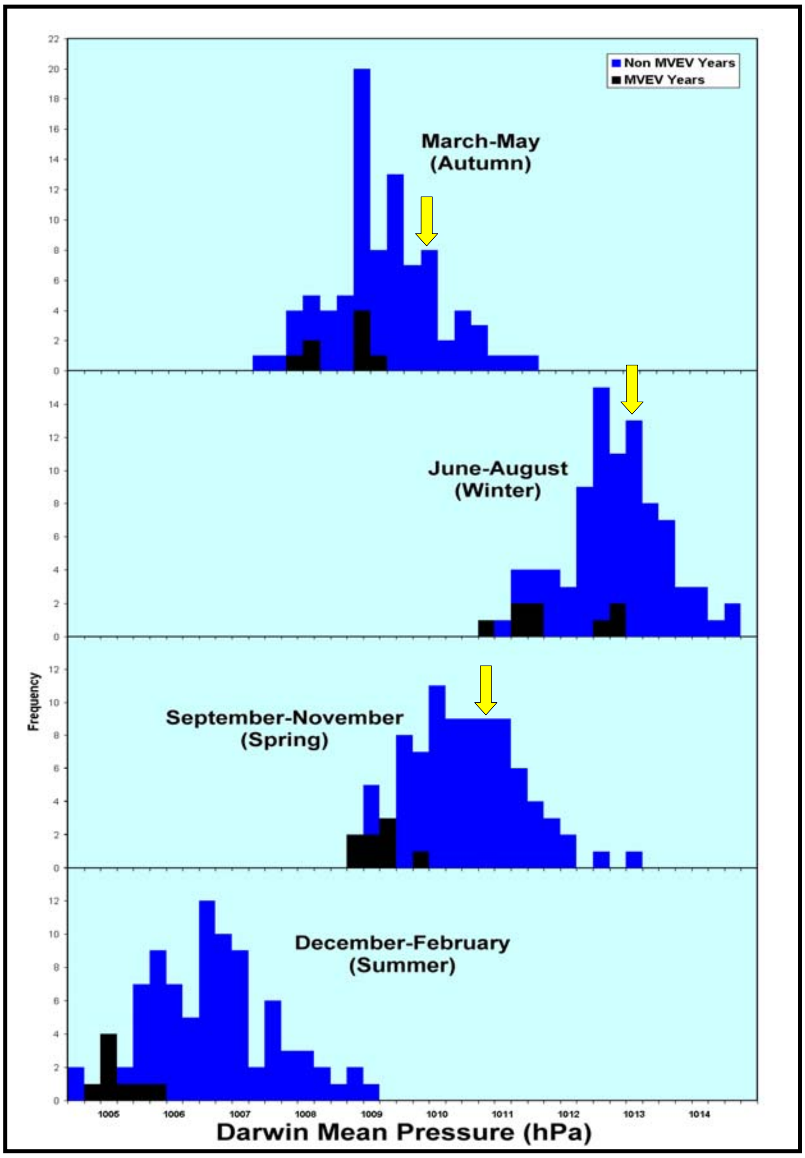


Figure 6. The SO by seasons prior to MVEV active years, according to Nicholls (1986), updated up to November 2004. The black bars represent the pre-MVEV active seasons. The yellow arrows indicate the respective seasons SO values relevant to the 2003-2004 season.

- The Lachlan, Murrumbidgee & Murray River systems,
- The Northern Rivers (that lead to the Gulf of Carpentaria), and
- The North Lake Eyre system.

The hypothesis states that if rainfall levels in these four catchment basins are equal to or greater than decile 7 for either the last quarter of the previous year (eg. October-December 2002) or the first quarter of the current year (January-March 2003) and the last quarter of the current year (October-December 2003), then a MVEV outbreak is probable.

Rainfall was not above decile 7 for all the catchment basins for the last quarter of 2002, the first quarter of 2003 or the last quarter of 2003. Thus, Forbes' hypothesis was not satisfied for the 2003-2004 season. As rainfall was also not above decile 7 in all the catchments for the first quarter of 2004, Forbes hypothesis would suggest that an MVEV outbreak in southeastern Australia would be unlikely for the 2004-2005 season.

Nicholls' hypothesis uses the Southern Oscillation (SO) as a tool to indicate a possible MVEV epidemic. He noted a correlation between past outbreaks of MVEV and the SO (as measured by atmospheric pressures at Darwin in mm) for the autumn, winter and spring period prior to a disease outbreak. For the autumn, winter and spring periods of 2003, the SO values of 1010.13mm, 1013.10mm and 1010.70mm respectively (indicated on Figure 6 by the yellow arrows), were all outside the range of values for the same period of past MVEV outbreak years (Figure 6). Likewise, the summer 2003–2004 SO value of 1006.63mm was also much higher than that experienced during MVEV years. Currently, the autumn, winter and spring values according to Nicholls' for 2004 are 1009.20mm, 1013.93mm and 1011.27mm, respectively. While the autumn figure is in the range of past MVEV outbreak years, the winter and spring values are not.

MOSQUITO MONITORING

Methods

Mosquitoes were collected overnight in dry-ice baited Encephalitis Vector Surveillance type traps. They were then sent live in cool, humid Eskies via overnight couriers to the Medical Entomology Unit, Centre for Infectious Diseases and Microbiology (CIDM), Institute of Clinical Pathology and Medical Research (ICPMR), Westmead for identification and processing for arbovirus isolation. The mosquitoes were identified via taxonomic keys and illustrations according to Russell (1993, 1996), Dobrotworsky (1965) and Lee *et al*. (1980 – 1989). A brief description of the main mosquito species for NSW appears in Appendix 2.

Mosquito abundances are best described in relative terms, and in keeping with the terminology from previous reports, mosquito numbers are depicted as:

- 'low' (<50 per trap),
- 'moderate' (50-100 per trap),
- 'high' (101-1,000 per trap),
- 'very high' (>1,000 per trap), and
- 'extreme' (>10,000 per trap).

All mosquito monitoring results (with comments on the collections) were placed on the NSW Arbovirus Surveillance Web site, and generally were available within 1-2 days of receiving the sample into the laboratory. Access to each location's result is from: **http://www.arbovirus.health.nsw.gov.au/areas/arbovirus/results/results.htm**.

Results

Overall, 132,584 mosquitoes representing 47 species were collected in NSW during the 2003-2004 season. *Culex annulirostris* and *Anopheles annulipes* were the most abundant and most important of the inland mosquito species during the summer months. *Ochlerotatus vigilax*, *Culex annulirostris*, *Culex sitiens, Coquillettidia linealis* and *Ochlerotatus notoscriptus* were the most numerous species on the coast. A full summary of the results on a location by location basis is included in Appendix 1.

Inland

Mosquito populations across the inland continued to be well down with the drought conditions and the total collected was one of the lowest recorded by the Arbovirus Surveillance Program. A total of 37,787 mosquitoes, comprising 17 species was collected from inland NSW. *Culex annulirostris* was the dominant species trapped at most sites and comprised 72% of the total inland collections. *Anopheles annulipes* (18%) was the next most common species.

Coastal

Only five locations undertook trapping from the coast. Mosquito numbers were up by about 50% upon the previous season, largely due to the big collections made at Heatherbrae, Port Stephens. This was probably due to the above average rainfall through January and February. In total, 83,138 mosquitoes comprising 43 species were collected from coastal NSW. The most common species collected were *Ochlerotatus vigilax* (45% of the total coastal mosquitoes trapped), *Culex annulirostris* (15%), *Culex sitiens* (9%) and *Coquillettidia linealis* (8%).

Metropolitan Sydney

Mosquito collections from Sydney were relatively low with the dry conditions. A total of 11,659 mosquitoes, comprising 22 species, was collected from metropolitan Sydney. *Ochlerotatus vigilax* (49% of the total Sydney mosquitoes trapped) was the most common species followed by *Ochlerotatus notoscriptus* (29%) and *Culex annulirostris* (10%).

A brief summary of the surveillance for each location follows the Sentinel Chicken Flock section. Note that complete mosquito monitoring results are available on the NSW Arbovirus Surveillance web site.

ARBOVIRUS ISOLATIONS FROM MOSQUITOES

http://www.arbovirus.health.nsw.gov.au/areas/arbovirus/about/methods.htm

Methods

Only mosquitoes collected from the inland and some Sydney sites were processed for viruses. Viral isolation methods were as per earlier annual reports (Doggett *et al*., 1999a, 2000). Assays were used to identify any suspected viral isolate, and can identify the alphaviruses - BFV, RRV and Sindbis (SINV), and the flaviviruses - MVEV, KUNV, Alfuy (ALFV), Edge Hill (EHV), Kokobera (KOKV) and Stratford (STRV). Any isolate that was not identified by the assays was labelled as 'unknown'.

Positive results were sent to Dr Jeremy McAnulty, Director, Communicable Diseases Branch, NSW Health, to the relevant Public Health Unit, and posted on the NSW Arbovirus Surveillance Web Site (under 'Mosquito/Chicken Results') and under each locations' surveillance results.

Results

http://www.arbovirus.health.nsw.gov.au/areas/arbovirus/results/virusisolates.htm

Of the mosquitoes processed, there were 13 viral isolates. These are listed in Table 1.

Table 1. Arbovirus Isolates, 2003-2004.

LOCATION - Site	Date Trapped	Mosquito Species	RRV	EHV	KOKV	Virus?	TOT
BOURKE - STW	11/Dec/03	*Culex annulirostris*		1			**1**
BOURKE - STW	9/Feb/04	*Anopheles amictus*				1	**1**
BOURKE - STW	10/Feb/04	*Culex annulirostris*			2		**2**
BOURKE - STW	18/Feb/04	*Culex annulirostris*			1		**1**
MENINDEE – Darl R	3/Mar/04	*Culex annulirostris*	2				**2**
BOURKE - STW	4/Mar/04	*Culex annulirostris*			1		**1**
BOURKE - STW	5/Apr/04	*Culex annulirostris*				4	**4**
BOURKE - STW	5/Apr/04	*Anopheles amictus*				1	**1**
TOTAL			**2**	**1**	**4**	**6**	**13**

RRV = Ross River virus, EHV = Edge Hill virus, KOKV = Kokobera virus, Virus? = unknown (not MVEV, KUNV, EHV, STRV, KOKV, RRV, BFV or SINV)

SENTINEL CHICKEN PROGRAM

http://www.arbovirus.health.nsw.gov.au/areas/arbovirus/about/chickenmethods.htm

Location of flocks

Generally, the monitoring season begins in late spring and ends in early autumn. Flocks are located in known or suspected 'hot spot' areas for MVEV and KUNV. The 2003-2004 season began on November 10^{th} 2003 and ended on April 28^{th} 2004. For 2003-2004, six flocks each containing 15 Isa Brown pullets were deployed at Griffith, Leeton, Menindee, Macquarie Marshes, Bourke and Lake Cargelligo (Figure 1).

Methods

The NSW Chicken Sentinel Program was approved by the WSAHS Animal Ethics committee. This approval requires that the chicken handlers undergo training to ensure the chickens are cared for appropriately and that blood sampling is conducted in a manner that minimises trauma to the chickens. The chickens are cared for and bled by local council staff and members of the public. Laboratory staff, under the supervision of a veterinarian, are responsible for training the chicken handlers. A veterinarian (usually the Director of Animal Care at Westmead, Dr Ross Mathews) must inspect all new flock locations prior to deployment to ensure animal housing is adequate. Existing flocks are inspected approximately every two years. The health of each flock is reported weekly, and is independently monitored by the Animal Ethics Committee via the Director of Animal Care.

All flocks are bled weekly throughout the season. Since 2002 whole blood has been collected directly on to absorbent Nobuto papers (manufactured by Advantec MFS, Inc. Pleasanton, CA 94588-3335. USA) In brief, a small cut is made in the wing vein using a blood lancet, the Nobuto paper is applied directly to the wound and blood is adsorbed into the paper strip. Strips are allowed to air dry before they are wrapped in paper and posted to the laboratory. This method has significantly reduced the time taken to collect samples and the amount of blood taken from each chicken from 2ml to 0.1ml. The method has removed the need for a cold chain, centrifugation and separation of serum from blood cells. As samples are posted to the laboratory there has been a significant saving in courier and consumable costs from approximately $25 per week per flock to $1.45 per week per flock. However, the laboratory workload has increased slightly due to the need to elute the blood samples from the paper.

The method was modelled on the system used by the Centers for Disease Control and Prevention (CDC) USA since 1979 (Pierre Rollin, *pers. comm.*). It was standardised and evaluated retrospectively and prospectively over a two-year period before introducing it into routine use in the 2002/2003 season.

The eluates are tested by “defined epitope” blocking enzyme-linked immunoassays (ELISAs), for MVEV and KUNV antibodies. These ELISAs had been originally developed, standardised and evaluated against the “gold standard” neutralisation, using serum, in 1996. They were restandardised and evaluated retrospectively and prospectively, against neutralisation, using eluates, in 2000 (Linda Hueston, unpublished data; report available on request).

Antigens used in the ELISAs are purified whole virions, the conjugates consist of purified horseradish peroxidase (HRP)-labelled monoclonal antibodies (against defined viral epitopes) prepared in-house. Testing time is 2hrs 15 minutes.

Positive samples are repeated in titration series in parallel with the previous week’s bleed. The next weekly bleed is run in parallel with these samples in titration series. Neutralisation tests are set up to confirm ELISA positives. A definitive result is one which demonstrates a four-fold or greater rise in ELISA titre between consecutive samples, with confirmation by neutralisation.

Results are disseminated via email to the relevant government groups as determined by

NSW Health and are placed on the NSW Arbovirus Surveillance website. Confirmed positives are notified by telephone to NSW Health and Communicable Diseases Network, Australia (CDNA).

Results

The season began with 90 pullets in groups of 15 at 6 locations in NSW. Five chickens died during the season, due to heat stress. A total of 1,768 samples were received from the 6 flocks in NSW over a six-month period in 2003-2004. This represented 3,536 ELISA tests (excluding controls and quality assurance samples), with each specimen being tested for MVEV and KUNV antibodies

There was one seroconversion to MVEV at Menindee in December 2003. Full results are in Table 2.

Table 2. Results for Menindee MVEV positive chicken, 2003-2004.

Date of collection	MVEV ELISA titres$^+$	KUNV ELISA titres$^+$	MVEV neutralisation titres *	KUNV neutralisation titres *
04/12/03	Neg (<10)	Neg (<10)	<10	<10
17/12/03	Neg (<10)	Neg (<10)	<10	<10
23/12/03	Pos (20)	Neg (<10)	<10	<10
31/12/03	Pos (320)	Neg (<10)	1280	<10
24/02/04	Pos (320)	Neg (<10)	320	<10

* using a 90% endpoint

As a result of this seroconversion, the Communicable Disease Control Branch of the South Australian Health Department, in collaboration with the South Australian Department of Primary Industries, undertook opportunistic testing of production chickens at Paringa near Renmark. The samples were collected on 9th March 2004 and submitted to the Arbovirus & Emerging Diseases Unit at CIDM, ICPMR for testing. Forty-four samples from chickens varying in age from 32 to 45 weeks were submitted. One of the 32-week-old chickens returned an ELISA total antibody and IgM positive result for MVEV antibody only, which was confirmed by neutralisation. Follow-up testing was not possible as these were production chickens not sentinels.

No cases of MVEV disease were detected in humans in NSW, Victoria or South Australia in 2003-2004. We confirmed a diagnosis of MVEV in a child from the Tennant Creek area in the Northern Territory in April 2004, on behalf of NT Health.

HUMAN NOTIFICATIONS

http://www.arbovirus.health.nsw.gov.au/areas/arbovirus/human/human.htm

Tables 2 and 3 contain the number of laboratory notifications of human RRV and BFV disease by Area Health Service (AHS) for NSW. Note that these are laboratory notifications based on a single IgM positive specimen, and may not always represent infections from this season, as IgM may persist for long periods.

The total number of alphavirus notifications for the period July 2003 to June 2004 (Figure 7) was 988 (including 641 RRV, 347 BFV) and this was slightly below the average for the previous five seasons of 1022, although higher than the ten-year average of 926 notifications. The coastal region accounted for 786 (80%) of the BFV and RRV notifications, which is around the average (755) for the previous five seasons. The 202 notifications (20%) from the inland were over double that of the previous two seasons, although still well below the previous five seasonal average of 262. Within the Sydney region there were 46 cases reported, almost half the five-season average.

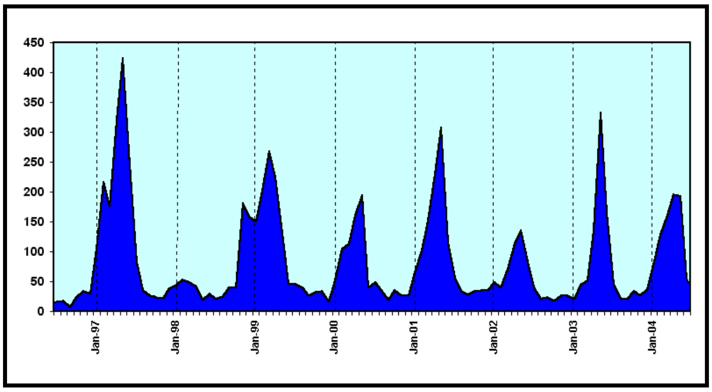


Figure 7. Reports of human cases of arbovirus infections by month in NSW, July 1997 - June 2004.

The Northern Rivers Area Health Service received the highest number of notifications (295) with the Mid-North Coast Area Health Service having 284 reports. Combined, these two areas accounted for almost 60% of all the arbovirus notifications for the state.

Table 2. Ross River virus disease notifications according to Area Health Service, July 2003 - June 2004.

Month	CS	NS	WS	WE	SW	CC	HU	IL	SE	NR	MN	NE	MA	MW	FW	GM	SA	Total
Jul	0	0	0	0	0	0	1	1	0	10	2	0	1	0	0	0	0	**15**
Aug	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	**5**
Sep	0	0	0	0	0	0	1	0	0	2	0	0	1	0	0	2	1	**7**
Oct	0	0	0	0	0	0	2	0	0	3	2	0	0	0	1	0	2	**10**
Nov	0	0	0	0	0	1	0	0	0	5	3	0	1	0	2	0	1	**13**
Dec	0	0	1	0	0	0	0	2	0	4	3	0	1	2	1	3	2	**19**
Jan	0	3	0	1	0	1	3	3	1	3	4	7	3	4	2	6	1	**42**
Feb	0	4	2	2	1	0	5	5	0	4	9	33	12	4	6	9	2	**98**
Mar	1	3	2	2	1	1	8	1	1	34	17	24	4	4	6	8	2	**119**
Apr	2	3	2	1	1	7	24	0	6	53	30	9	3	1	2	1	2	**147**
May	0	0	0	0	0	5	25	2	0	45	50	5	0	1	2	1	1	**137**
Jun	0	1	0	0	0	0	3	1	0	6	15	1	0	1	0	0	1	**29**
Total	**3**	**14**	**7**	**6**	**3**	**16**	**72**	**15**	**8**	**170**	**135**	**80**	**26**	**17**	**22**	**31**	**16**	**641**

CS = Central Sydney, NS = Northern Sydney, WS = Western Sydney, WE = Wentworth, SW = South Western Sydney, CC = Central Coast, HU = Hunter, IL = Illawarra, SE = South Eastern Sydney, NR = Northern Rivers, MN = Mid North Coast, NE = New England, MA = Macquarie, MW = Mid Western, FW = Far Western, GM = Greater Murray, SA = Southern Area.

Table 3. Barmah Forest virus disease notifications according to Area Health Service, July 2003 - June 2004.

Month	CS	NS	WS	WE	SW	CC	HU	IL	SE	NR	MN	NE	MA	MW	FW	GM	SA	Total
Jul	0	0	0	1	0	0	1	0	0	19	7	1	0	0	0	0	0	**29**
Aug	0	0	0	0	0	1	1	0	0	8	5	0	0	0	0	0	0	**15**
Sep	0	0	0	0	0	0	2	0	0	5	6	0	0	0	0	0	0	**13**
Oct	0	0	0	0	0	0	1	2	0	12	9	0	0	0	0	0	0	**24**
Nov	0	0	0	0	0	0	1	0	0	7	5	0	1	0	0	0	0	**14**
Dec	1	0	1	0	0	1	1	1	0	4	4	0	0	0	0	3	2	**18**
Jan	1	0	0	0	0	0	1	2	0	14	10	2	0	0	1	2	1	**34**
Feb	0	1	0	1	0	0	0	2	0	11	10	1	0	0	0	2	2	**30**
Mar	0	0	0	0	0	0	4	0	0	14	16	2	1	0	1	2	1	**41**
Apr	0	1	0	0	0	1	4	0	0	16	21	4	0	0	0	0	1	**48**
May	0	0	0	0	0	0	4	0	0	10	40	1	0	0	0	1	0	**56**
Jun	0	0	0	0	0	0	2	0	0	5	16	1	0	0	0	0	1	**25**
Total	**2**	**2**	**1**	**2**	**0**	**3**	**22**	**7**	**0**	**125**	**149**	**12**	**2**	**0**	**2**	**10**	**8**	**347**

DISCUSSION

The dry conditions that began in the summer of 2000-2001 continued through into the 2003-2004 season. Rainfall was slightly above average in the second half of 2003 for southern inland NSW, however the first quarter of 2004 was exceptionally dry. Not surprisingly, mosquito numbers across the inland were again low, with minimal arboviral activity as shown by the few mosquito isolates and below average notifications of human arboviral disease.

Despite the low activity, there was one seroconversion to MVEV in the sentinel chicken flock in Menindee in December 2003, following a period of localised rainfall. Subsequently, opportunistic sampling of South Australian chickens in the Renmark area showed MVEV infection in a production chicken. Since the chicken was only 32 weeks old, and was also IgM positive it is assumed that it was infected during the summer of 2003-2004. Both of these incidents predated seroconversions in northern Australia by several months.

There are two schools of thought regarding MVEV in southern Australia. Traditionally, it was thought that MVEV is introduced into NSW from endemic regions of the northwest, via Central Australia, carried by migrating birds, following consecutive seasons of heavy rainfall (Marshall 1988). An alternative theory is that MVEV persists in small enzootic foci within the southern states.

There have been reports of MVEV and KUNV activity in sera of feral pigs collected from widely separated areas of northern and central NSW, indicating inter-epidemic MVEV infection (Gard *et al*. 1976). Prior to 2000-2001, there had been no known MVEV infections in southeastern Australia since 1974. If the first theory were correct, nobody born after 1974 should have MVEV antibody, unless they had travelled to endemic areas. This is consistent with the results of serosurveys in the Murrumbidgee area in the 1980's by Hawkes *et al*. (1985) and by our laboratory during the 1990's (L. Hueston, *unpublished data*).

However, in a small serosurvey in the Macquarie Marshes area in early 2001, 46% of 35 subjects had MVEV antibodies; of these two had been born in the area after 1974 but had not travelled to northern Australia (L. Hueston, *unpublished data* – reports of both serosurveys are available on request). A serosurvey of companion animals also detected MVEV antibody in young animals (Peter Kirkland, Elizabeth Macarthur Institute, *personal communication*). These recent findings and the chicken MVEV seroconversion at Menindee are consistent with an enzootic pattern and/or overwintering of MVEV in the mosquito population (especially in floodwater, *Ochlerotatus* spp.) following the 2000-2001 activity, as has been previously described (Broom *et al*. 1995).

The 2002-2003 season was one of the driest ever recorded for the NSW inland, and mosquito numbers and arbovirus activity were the lowest observed for the history of the program. Likewise, winter and spring of 2003 were also not particularly wet. Thus it would appear unlikely that migrating birds had introduced the virus.

Menindee has very large shallow lakes and is an irrigation area. It has abundant wildlife, particularly water birds. In this setting it would not be surprising if MVEV were endemic/enzootic in small areas. This recent MVEV activity in NSW adds evidence supporting this view and emphasises the need for a more detailed study.

Unfortunately, no mosquito collections were undertaken during the time of the seroconversion and thus there is no evidence to support or refute either of these speculations with respect to 2003-2004. It is worth noting that the mosquito collections at Menindee, which began in response to the MVEV seroconversion were quite high for the location. Whether these numbers are linked to the growing local irrigation practices or some other factor is unknown. In light of the MVEV activity over recent years, it would seem prudent that both mosquito and sentinel chicken surveillance are maintained at Menindee.

The low rainfall in Sydney meant that mosquito numbers were down this year; around 25% less were collected this season compared to the last, mainly due to fewer *Ochlerotatus vigilax* being collected. There were no isolates from the mosquitoes and the number of human notifications within the Sydney Region (7 BFV and 41 RRV) was almost half the five-season average.

Coastal trapping continued to be limited, this year to five locations, and again there were no sites from the south coast. Weather conditions over the season were generally dry along the coast, although punctuated in some areas during February with localised heavy precipitation. This led to some large collections at Port Stephens resulting in an overall increase in mosquito collections this season compared to the last. It would appear however, that this mosquito activity was confined to certain regions. For example, numbers along the central coast did not rise substantially. Following the February rainfall, both BFV and RRV were again active along the north coast with 274 notifications of BFV and 305 of RRV from the Mid North Coast and the Northern Rivers AHSs. When combined (579 total), these were almost 60% of all BFV/RRV reports from the state. These AHSs have produced around half of all the disease notifications for the state over the last four seasons (Table 4).

Table 4. Notifications of BFV & RRV disease from the Northern Rivers & Mid North Coast AHSs, over the last four mosquito seasons.

Virus	2000-2001	2001-2002	2002-2003	2003-2004	Total
BFV	307	250*	350	274	**1181**
RRV	215	82	347	305	**949**
Total	**522**	**332**	**697**	**579**	**2130**
NSW Total	**1161**	**711**	**901**	**988**	**3761**

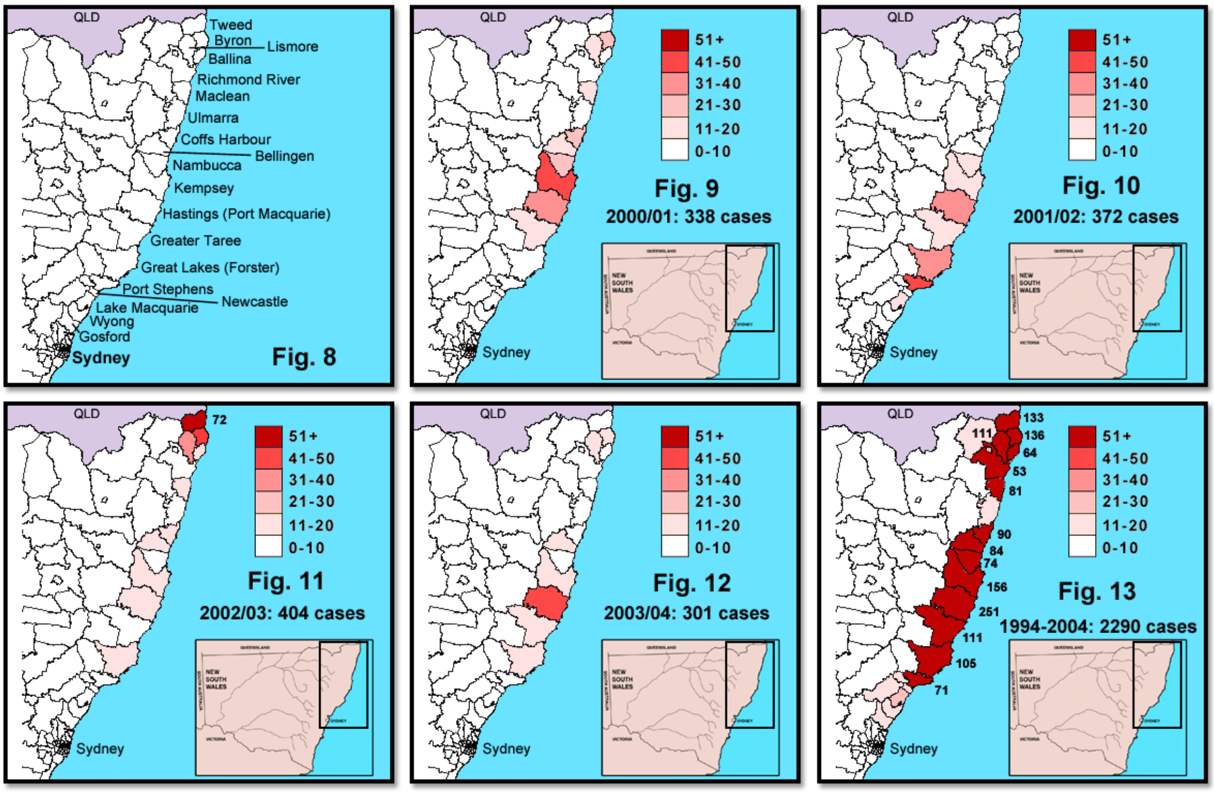
The BFV activity for 2003-2004 was the fourth consecutive annual outbreak for the north coast. As noted in the previous report (Doggett *et al*. 2003), outbreaks of BFV disease in humans prior to 2000 had been confined to a single season, with little activity in the following year (Doggett *et al*. 1999b). This is very different to what has been observed during 2000-2004, with continuing major activity for the coast of NSW north of Sydney. These four outbreaks did however show some differences. In 2000-2001, activity was mainly confined to the Kempsey region on the mid-north coast and followed upon heavy flooding as a result of record rainfall, along with high spring tides. The 2001-2002 activity was more diffuse along the coast, with the first ever cases being reported in western Sydney. However, the mid-north coast region again experienced the largest number of cases. It is suggested that this outbreak may have had a greater involvement of freshwater vectors as there were several isolates from species such as *Cx. annulirostris*, *Oc. notoscriptus* and *Oc. procax* (Doggett *et al*. 2002). As these vectors vary in their habitat and geography, there may have been a demographically different human population exposed to the virus in the 2001-2002 outbreak compared with the prior season. For 2002-2003, the far north coast had most activity, although the mid-north coast had 136 cases and for the 2003-2004 season, the mid-north coast was again the most active BFV region. For all outbreaks, there was late summer rainfall, which undoubtedly contributed to vector abundance and virus activity, but why BFV has been continually active and why the mid-north coast of NSW has been the 'hot-spot' over the last four years is unknown (Doggett and Russell 2005). Clearly, the disease must be considered to be an emerging problem in NSW, and warrants further investigation.

It is interesting to examine the changing face of the epidemiology of BFV in NSW, by comparing the most recently published account for NSW, which covered the period 1995-1999 (Muscatello and McAnulty 2000), with the data presented herein. While the Muscatello and McAnulty paper listed notifications per calendar rather than fiscal year, the notification rates can be compared. They observed that the highest rates of BFV disease were found in the Mid North Coast AHS and Northern Rivers AHS, with average annual rates of 28.2/100,000 and 22.0/100,000, respectively. However, for the last five seasons, the rates are 57.2 and 40.0 (Doggett and Russell 2005), i.e. around double those previously reported. This further highlights the emerging disease status of BFV for the northern coastal region.

To further examine this recent trend of BFV activity for the north coast and to demonstrate localities of greatest risk, the disease notifications of BFV per Statistical Local Area (SLA) over the four consecutive outbreaks and combined for the period 1994/95 – 2003/04 are presented in Figures 8-13. The highest number of BFV disease notifications occurred in the Hastings SLA (Port Macquarie being the major city), with 251 cases and almost continual activity from 2000-2004. The next highest had almost

100 cases less, some 156, in the adjoining SLA of Kempsey. These figures show that Port Macquarie and its immediate surrounds are the current 'hot-spot' for BFV activity in NSW, although why this should be so is unknown. Within this region mosquito species that are transmitting BFV are unknown, likewise there the native vertebrate hosts for the virus are yet to be identified. This lack of information means that it is difficult to accurately target disease reduction efforts, thus the human population in the region will be continually at risk from this emerging disease as it has been over the last four seasons.

THE NEW SOUTH WALES ARBOVIRUS SURVEILLANCE WEB SITE

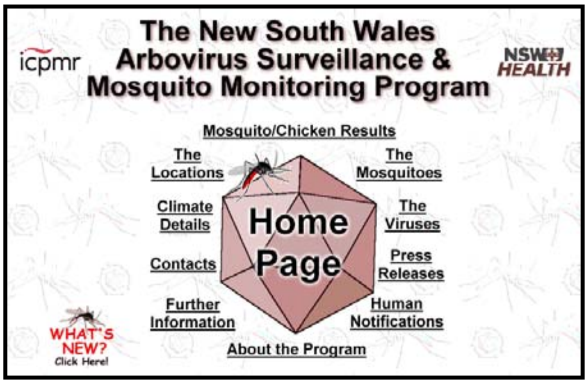


Figures 8-13. **BFV notifications for the NSW North Coast. Fig 8.** List of Statistical Local Areas (SLA), the name in the bracket is the largest urban centre for the area, if that name is different to the SLA name. **Fig 9.** BFV notifications for 2000-2001. **Fig 10.** BFV notifications for 2001-2002. **Fig 11.** BFV notifications for 2002-2003. **Fig 12.** BFV notifications for 2003-2004. **Fig 13**. BFV combined notifications for the 10 seasons of 1994-2004. **Note that the numbers on Figs. 11 & 13 show the cases of BFV disease when >51 were reported**.

http://www.arbovirus.health.nsw.gov.au/

The NSW Arbovirus Surveillance web site was established in early 1999 to facilitate the rapid dissemination of surveillance results (Doggett *et al.*, 1999c). An additional important function is to provide information on mosquitoes and the arboviruses they transmit. Over the last year, the site has continued to grow to the current size of 155MB, and has 1,140+ pages of information.

Added to the site since the last annual report include:



- Archived data for the 2003-2004 season.
- Weekly rainfall summaries,
- Monthly rainfall summaries, with long-term averages,
- Monthly rainfall and temperatures maps,
- Monthly SOI updates,
- An increase photographic collection of adult & larval mosquitoes,

As of January 2003, statistics on the number of 'hits' (i.e. visits to the web site) were obtained (Table 4). As of July 2004, the web site is averaging around 23,000 hits/month, or extrapolated, around 280,000 annually.

Table 4. Number of hits to the NSW Arbovirus Surveillance Program web site, July 2003 – June 2004.

Month	Jul-03	Aug-03	Sep-03	Oct-03	Nov-03	Dec-03
Hits	18,577	17,582	20,359	19,593	23,379	19,619

Month	Jan-04	Feb-04	Mar-04	Apr-04	May-04	Jun-04	Total
Hits	22,847	21,866	28,169	28,380	28,714	28,160	**277,245**

Appendix 1. LOCATION-BY-LOCATION SUMMARY

http://www.arbovirus.health.nsw.gov.au/areas/arbovirus/results/results.htm

Inland Locations

Albury: collections were well below average for most of the trapping season and 'high' on four occasions only, in all cases from the Sewerage Treatment Works trapping site. No viruses were isolated.

Bourke: mosquito numbers were mostly 'low' to 'medium' up until February and thereafter were consistently 'high' until the end of the season. One 'very high' collection was made in early February, being dominated by *Culex annulirostris*. There were 11 viral isolates; 1 EHV from *Culex annulirostris* trapped on 11/Dec/2003, 2 KOKV from *Culex annulirostris* on 10/Feb/2004, 1 KOKV each from *Culex annulirostris* trapped on 18/Feb/2004 and 4/Mar/2004, and 6 unknowns (Table 1). No seroconversions to MVEV or KUNV occurred in the sentinel chickens.

Condobolin: no mosquito collections were made or sentinel chickens operated this year.

Forbes: some trapping was undertaken through March and April, with only 'low' mosquito numbers ensuing.

Griffith: as per the season of 2002-2003, mosquito numbers were well below average through the entire 2003-2004 season with no 'very high' collection being made. There were no viral isolates or any seroconversion to MVEV or KUNV in the sentinel chickens.

Lake Cargelligo: no mosquito collections were undertaken this season and there were no seroconversions to MVEV or KUNV in the sentinel chickens.

Leeton: the highest numbers from any inland trapping location this season were again yielded from Leeton. Farm 347 had several 'very high' collections through January and February, while in the other months collections were mostly 'high'. Almond Road had 'high' numbers continuously from early December to April. There were no viral isolates or any seroconversion to MVEV or KUNV in the sentinel chickens.

Macquarie Marshes: no mosquito trapping was undertaken this season. No seroconversions to MVEV or KUNV occurred in the sentinel chickens.

Menindee: there was one seroconversion to MVEV in a sentinel chicken on the 23/Dec/2003. In response to this detection, mosquito trapping began in late February, where mosquito numbers were thereafter 'high' to 'very high' and dominated by *Culex annulirostris*. From the 3/Mar/2004, there were two isolates of RRV, both from *Culex annulirostris*.

Tamworth: no mosquito collections were undertaken this season.

Coastal Locations

Ballina: mosquito numbers were below average up until February, although December had some notable collections of the saltmarsh breeding mosquito *Culex sitiens*. Thereafter numbers were ‘high’ and even ‘very high’ through March at North Creek Road. *Verrallina funerea* and *Culex sitiens* were the dominant species at this site. Greenfield Road tended to collect fewer mosquitoes, even though numbers were always ‘high’ or greater from February to the end of the trapping season. The one ‘very high’ collection for this site occurred in late March and was dominated by the freshwater marsh breeding mosquito *Coquillettidia variegata*. No virus isolation was undertaken.

Batemans Bay: no mosquito collections were made this year.

Gosford: as is often the case for Gosford, mosquito collections were variable throughout the season with collections numbering between ‘low’ and ‘high. This was due to fluctuations in the dominant species, *Ochlerotatus vigilax*, with breeding of this species following high spring tides. No virus isolation was undertaken.

Port Stephens: a similar trend to the last season was again observed with the collections from the sites varying substantially in mosquito abundance and species composition. This reflects the diverse mosquito breeding habitats within the region, with some trapping sites being near freshwater habitats, while others are near saltmarsh environments. Gan Gan had mostly ‘high’ collections throughout the season, with *Coquillettidia linealis* and *Coquillettidia variegata* dominant. Numbers from this site were mostly around average. Mosquito numbers at Saltash were up, and were ‘high’ throughout most of the season with *Ochlerotatus vigilax* dominating. Medowie yielded ‘high’ numbers from January until the end of the season, with two ‘very high’ collections in late January/early February. The main mosquito species trapped at Medowie were *Ochlerotatus vigilax* and *Culex annulirostris*. As always, Karuah was strongly dominated by *Ochlerotatus vigilax* and collections were mostly ‘high’ throughout the season. Heatherbrae continued to trap the most mosquitoes for any site within NSW, with mosquito numbers being mainly ‘very high’ from late November to early April. *Ochlerotatus vigilax* was the most common species trapped at Heatherbrae, although freshwater species including *Culex annulirostris* and *Coquillettidia linealis* were also trapped in ‘high’ numbers. No virus isolation was undertaken.

Tweed Heads: collections were mostly ‘low’ and below average, with one ‘high’ trap number in February from the Beltana Road site, which was dominated by *Culex sitiens*. No virus isolation was undertaken.

Wyong: trapping yielded mostly ‘low’ numbers, with the occasional ‘medium’ collection, with *Ochlerotatus notoscriptus* being the main species captured. No virus isolation was undertaken.

Sydney Locations

Blue Mountains: only four collections were made this season, with mostly 'low' mosquito numbers being trapped. The two 'medium' collections from Glenbrook Lagoon were dominated by *Ochlerotatus notoscriptus*. Virus isolation was undertaken from the mosquitoes but no isolates were yielded.

Concord: mosquito numbers were mostly 'low' interspersed with some 'medium' collections. The Powell's Creek site yielded the only 'high' numbers, which were dominated by *Ochlerotatus vigilax*. No virus isolation was undertaken.

Hawkesbury: no mosquito collections were undertaken this season.

Parramatta: mosquito numbers were down this year and the collections varied markedly between sites. As George Kendall Reserve is situated the closest to breeding grounds of *Ochlerotatus vigilax*, this site consistently yielded the highest collections. Generally, numbers were 'medium' towards the start and end of the season and 'high' from late January to early March. The only other site that had numbers 'medium' or higher was Eric Primrose Reserve, which had three 'high' collections between January and mid-February. Virus isolation was undertaken from the mosquitoes but no isolates were yielded.

Penrith: only limited collections were made with traps set on four occasions. Mosquito collections were all 'low' in number. Virus isolation was undertaken from the mosquitoes but no isolates were yielded.

Ryde: the majority of the trapping yielded 'low' to 'medium' mosquito densities. Maze Park had some 'high' numbers from December to February, largely due to breeding of *Ochlerotatus notoscriptus*. Numbers of *Ochlerotatus vigilax* were down upon the previous season. Only the Lambert Park collections were processed for viruses and none were isolated.

Appendix 2. THE MOSQUITOES

The following briefly details the main mosquito species collected in NSW.

	***Anopheles annulipes*.** A mosquito collected throughout NSW, although is most abundant in the irrigated region of the Murrumbidgee where it can be collected in the 1000's. Despite its abundance, it is not thought to be a serious disease vector.
	The Common Marsh Mosquito, ***Coquillettidia linealis*.** Found throughout NSW but especially in areas with freshwater marshes such as the Port Stephens area. Both BFV & RRV have been isolated from this species and is probably involved in some transmission.
	The Common Banded Mosquito, ***Culex annulirostris*.** The species is common in the NSW inland regions that have intense irrigation. This species is highly efficient at transmitting most viruses and is responsible for the spreading of most of the arboviruses to humans inland.
	The Brown House Mosquito, ***Culex quinquefasciatus*.** A common species throughout Australia and tends to breed in polluted ground pools. While this species is an important nuisance biter, it appears to be a poor vector of most of the arboviruses.
	The Common Domestic Mosquito, ***Ochlerotatus notoscriptus*.** A common species that breed in a variety of natural and artificial containers around the home. It is the main vector of dog heartworm and laboratory studies shows it be an excellent transmitter both of RRV and BFV.
	The Saltmarsh Mosquito, ***Ochlerotatus vigilax*.** The most important species along coastal NSW. This species breeds on the mud flats behind saltmarshes and can be extremely abundant and a series nuisance biter. It is the main vector for RRV and BFV along the coast.

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